Research Article

Impact of Garlic (*Allium sativum*) and/or Ginger (*Zingiber officinale*) oils supplementation on the Growth Performance, physiological responses, and gene expression of Nile tilapia

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Abstract | A feeding trial for 4 weeks was performed to estimate the impacts of dietary garlic oil and/or ginger oil on the growth performance, physiological responses, and gene expression of Nile tilapia fish (*Oreochromis niloticus)*. A 180-healthy Nile tilapia having an average weight of 50.0±2.24 g was randomly distributed into 6 groups (30 fish/group/three replicates) as the following: T1 (control group) nourished with the basal diet only, T2 given the basal diet supplemented with 0.5% garlic oil, T3 given the basal diet supplemented with 1% garlic oil, T4 given the basal diet supplemented with 0.5% ginger oil, T5 given the basal diet supplemented with 1% ginger oil, and T6 given the basal diet supplemented with a mixture of 0.5% garlic and 0.5% ginger oil. The fish group received ginger oil in its diet at a 0.5% rate (T4) showed a significant increase of growth performance (overall body weight, weight gain, and specific growth rate) with a lower feed conversion ratio. Besides, garlic oil supplementation on fish diet at a 0.5% rate (T2) improved the overall body weight. The plasma biochemical parameters results indicate that garlic oil and/or ginger oil supplementation on the Nile tilapia diet at different levels raises markedly raises the T4 hormone level and reduces total cholesterol and LDL-cholesterol levels. Moreover, administration of 1% garlic oil and ginger oil (T3 and T5 respectively) and both of them (T6) to the diet of Nile tilapia significantly ($P<0.05$) reduces the plasma urea and uric acid concentration. The results of gene expression showed that TNF-α and IL-1β genes expression was the highest in the fish supplemented with 1% ginger oil (T5). It is concluded that garlic and/or ginger oil supplementation to the fish diet enhances growth as well as immunostimulant agents and improves the general health of Nile tilapia.

Keywords | Gene expression, Physiology, Garlic oil, Ginger oil, Nile tilapia.

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INTRODUCTION

Aquaculture is one of the significant monetary applications and vital sources of protein for human consumption (Hayatgheib et al., 2020). Fish is a an extremely nourishing food that contains various fundamental supplements like omega3, vitamin D, iodine, and calcium. Also, they are viewed as a source of profits in all countries (FAO, 2020). Nile tilapia (*Oreochromis niloticus)* is the 2nd aquaculture type in the world and has a high tolerance to environmental changes, so it is viewed as the principal candidate for creating aquaculture in unusual water sources (Brum et

al., 2017).

Different studies have reported that herbal products have already improved the immune status of tilapia fish (Jaali et al., 2020). Aqua-feed manufacturers hope to improve not only performance but also the immunity of cultured fish species (Hassaan et al., 2019). Natural immunostimulants are used as substitutes to antibiotics, vaccines, and other artificial complexes owing to its potential effect as an enhancer of the diseases' resistance by improving the innate immune system as well as increasing the digestive enzyme secretion, which promotes growth and increases fish survival rate (Van Hai, 2015). Marked considerations lately have been concerned to phytobiotics uses in aquaculture because it has numerous active principles such as essential oils, steroids, alkaloids, glycosides, terpenoids, flavonoids, phenolics, tannins, and saponins that enhance fish growth, stimulate immune parameters, and increase disease resistance (Ghosh et al., 2019). Widespread experiments have been intended to concentrate on the impacts of medicinal plants extracts and essential oils as immunostimulants in aquatic creatures (Sadek, 2020).

Garlic (*Allium sativum) is* considered as one of the greatest active ordinary immunostimulants, with a character of an "all-healing" herb (Lee and Gao, 2012). Garlic inclusion in fish and shrimp ration formulas markedly enhanced the growth performance, immunity and enhanced disease fighting (Agbebi et al., 2013). It contains many sulfur compounds which promotes its use as an antibacterial, antiviral, antifungal, antioxidant, antiprotozoal, and an active immuno-enhancer, growth supporter, and advances meat value (Mala et al., 2009). It has also been found to aggravate immune responses and reduce oxidative stress through increasing immuno-competence, improving motility of the gastrointestinal tract and modifying various enzyme activities that improve nutrient digestion and absorption (Hetta et al., 2014).

Ginger (Zingiber officinale) is perhaps of the most popular spices in the world, and its health benefits have also been widely used throughout the past. Gingerols, shogaols, and some associated phenolic ketone derivatives are the main energetic antioxidant compounds in ginger (Cao et al., 1993). Ginger has many biological actions in current years, such as antioxidants, anti-inflammatory, and antimicrobial activities (Siegel et al., 2014). Ginger improves immune status and rises growth in various fish types as *Oreochromis mossambicus* (Sadek, 2020), maintain tissue morphology and function, and strengthen tissue damage after tilapia niloticus infection (Brum et al., 2018).

The incorporation of extracts or essential oils into fish diet is the chief public technique for addition of medicinal plants products (Awad and Awaad, 2017). Combinations of different essential oils as feed supplements on fish diets are promising policies for efficient feeds and their efficiency (Salinas and Magadan, 2017). The most typical way to employ herbal oils and extracts in aquaculture is through the oral route of administration, while there were some reports which concluded that fish being bathed in medicinal plants' oils and extracts (Yilmaz and Ergun, 2012), mainly for the treatment of exogenous pathogens (da Cunha et al., 2018).

The current experiment was undertaken to assess the effects of garlic (*Allium sativum*) and/or ginger (*Zingiber officinale*) oil supplementation on the performance of growth, the expression of immune related gene, physiological responses, and general health of Oreochromis niloticus.

MATERIALS AND METHODS

Experimental fish

A 180-healthy Nile tilapia *(Oreochromis niloticus)* having a mean weight of 50.0±2.24 g was gotten from a fish breeding farm in Kafr El-Sheikh, Egypt. The fish were given two weeks to acclimate before the trial began. Fish were fed twice daily with tilapia floating crumbles diet containing 30% protein and 4000 Kcal gross energy (Aller Aqua Company, Egypt) during the accommodation period. Each aquarium received continuous ventilation from an electrical aquarium air pump. The mechanical filter eliminated organic waste materials. The water in each aquarium was routinely changed once a week throughout the study period. Temperature, dissolved oxygen, pH level, and total ammonia were all within the acceptable ranges for growing tilapia niloticus during the feeding study (Boyd, 1990). Once a week, the equipment and aquariums were cleaned. Fish were also routinely checked for activity and sickness.

Oil extraction

The oils of garlic and ginger gotten from the National Research Center, Dokki, Giza, Egypt. Oil extraction was achieved by the steam distillation technique (Brum et al., 2017) using Clevenger-like equipment. In a 1200 mL flask, 500 g of fresh garlic or ginger rhizomes were inserted. After submerging rhizomes in water, the heating mantle was activated. Distillation continued for 2 hours, then, the oil extract was obtained and kept in amber glass containers at 4 °C.

Experimental design and diet preparation

Fish were randomly stocked into 18 glass aquariums (80 L), with 10 fish in each aquarium and three replicates per group. Dietary treatments included T1 (control group): in which fish nourished with the basal diet only, T2: in which fish nourished the basal diet supplemented with 0.5% gar-

lic oil; T3: in which fish nourished the basal diet supplemented with 1% garlic oil, T4: in which fish nourished the basal diet supplemented with 0.5% ginger oil; T5: in which fish nourished the basal diet supplemented with 1% ginger oil, and T6: in which fish nourished the basal diet supplemented with a mixture of 0.5% garlic and 0.5% ginger oil. The basal diet was applied to all groups at 3% of body mass. The oil percentages (0.5% and 1%) were calculated per kilogram of diet, were added to the fish diets and mixed well, then kept at 4°C till used. The prepared investigational foods given twice a day at 9:00 am and 3:00 pm. The quantity of diet was bi-weekly accustomed rendering to the variation in body weight through the trial period, then oil supplementation was adjusted too. The experiment lasted for 4 weeks.

Growth Performance

All the fish/aquarium were weighted each 2 weeks throughout the trial. As the feeding experiment finished, weight gain (WG), feed intake (FI), specific growth rate (SGR), and feed conversion ratio (FCR) were calculated as designated by Jahanjoo et al. (2018).

Weight gain (Wg)	= Final weight – Initial weight
Feed intake (FI) (percent of biomass per day)	= (total feed offered / (W1 + W2)* / 2) / days) × 100
Specific growth rate (SGR)	= (logW2 - logW1) / (t2-t1) * x 100
Feed conversion ratio (FCR)	= Feed intake / weight gain

*W1 = initial weight, W2 = final weight. t2-t1 = feeding days.

Blood collection and biochemical analysis

As the trial finished, five fish from each replicate were taken randomly for blood analysis. Citrated syringes were used to collect the blood samples from the caudal blood vessels. The anticoagulated blood samples were centrifugated at 4000 rpm for 15 min to separate plasma, and the separated plasma was kept at -20°C till biochemical analysis as follows: The triiodothyronine (T3) and thyroxine (T4) levels measured by the radioimmunoassay method according to Wang et al. (2009). A lipid profile (triglycerides and total cholesterol) was performed through using auto analyzing kit (Biodiagnostic® kit, Egypt), using the suitable enzymatic procedures of Bogin and Keller (1987). Also, the plasma concentration of high-density lipoprotein-cholesterol (HDL) and low-density lipoprotein-cholesterol (LDL) was estimated colorimetrically through utilization of profitable kits produced by (Biodiagnostic® kit, Egypt) regarding to the method described by Bachorik and Ross, (1995) and Young, (2001) respectively. Kidney function tests (urea, uric acid, and creatinine) were measured regarding to producers' directions using popular kits (Biodiagnostic® kit, Egypt). Plasma urea levels were measured by means of Berthelot's reaction as recommended by Kaplan and Teng, (1982). The serum creatinine concentrations (mg/dl) were measured bestowing to Husdan and Rapoport, (1968), whereas the uric acid concentrations (mg/dl) were assayed bestowing to the technique described by Patton and Crouch, (1977). The plasma electrolyte profiles (calcium and phosphorus) were measured through utilization of marketable kits (Biodiagnostic® kit, Egypt) bestowing to the methods designated by Gindler and King, (1972) and Goodwin, (1970) respectively.

Tissue sampling, RNA Extraction and Quantitative Real-time PCR

As the experiment ended, spleen tissue samples were obtained from five fish/replicate and snap frozen in liquid nitrogen, then kept at trizol before storing at -80°C till used for RNA extraction and gene expression analysis. Total RNA was extracted from 50-100 mg of spleen tissue samples using TRI reagent (easy-RED™, iNtRON Biotechnology), according to the instructions of the standard protocol. The RNA integrity was judged by agarose gel electrophoresis. Also, the concentrations and purities of RNA were determined by measurement of absorbance at 260 and 280 nm using colorimeters and UV-visible spectrophotometer (biochrom® Ltd.). TOPscript™ cDNA reverse transcription kit (enzynomics, Korea) was used to synthesize the first strand cDNA from 2 µg of each total RNA sample bestowing to the producer's procedure. The primers of TNF-α and IL-1β genes were used to intensify the gene products for gene expression analysis, while the actin gene was utilized as a cleaning (internal standard) gene that was constant across the test groups (Table 1). qRT-PCR was finalized with the DTlite Real-Time PCR Detection System (DNA Technology, Russia) using SYBR Green with low ROX TOPreal™ qPCR 2X PreMIX (enzynomics, Korea) following the manufacturer's instructions.

Standard PCR circumstances were established up as follows: primary denaturation at 95°C for 10 min, followed by 40 rounds of denaturation 30 s at 95°C, annealing 30 s at 60°C, and extension 40 s at 72°C. After amplification, melting curve analysis was performed by heating from 65°C to 95°C, with 0.5°C incremental increases every 5 seconds to ensure specificity of qPCR products and verify the absence of primer dimers. The experimental samples and all genes were tested in duplicates, and each run contained a no temple control (NTC) and a RT negative (RT-) reaction. The fold change of mRNA expressions of TNF-α and IL-1β genes were evaluated using the $2^{-\Delta\Delta Ct}$ method as designated by Livak and Schmittgen, (2001).

Statistical analysis

The procedure of one-way ANOVA in SPSS version 25

Table 1: Primers used in gene expression analysis by qPCR.

Gene symbol	GenBank accession no.	Primer sequence (5′–3′)	Size (bp)
TNF-α	NM_001279533	F:5′GAGGCCAATAAAATCATCATCCC-3′ R: 5′- CTTCCCATAGACTCTGAGTAGCG-3′	161
IL-1β	KF747686.1	F: 5′- AAGGATGACGACAAGCCAAC-3′ R: 5′- CGCTGTGCTGATGTACCAGT-3′	174
β-actin	XM_003443127	F:5′- GTGCCCATCTACGAGGGTTA-3′ R: 5′- CTCCTTAATGTCACGCACGA-3′	156

Table 2: Effect of garlic and/or ginger oil on growth performance of Nile tilapia (means ± SE).

Items	Treatments					
	T1	T2	T3	T4	T5	T6
Body weight (g)						
Initial	50.01± 2.41	51.32±2.16	50.08±1.97	50.96±3.06	51.99±2.44	50.89±2.41
At 2nd w	65.92±2.96^{a}	65.60± 3.00^{a}	59.10±2.22^{b}	65.42±3.73^{a}	63.52±3.31^{a}	61.44±2.80^{b}
At 4th w	72.82±3.47^{ab}	75.53±3.29^{ab}	68.74±2.41^{b}	80.92±4.63^{a}	74.45±3.62^{ab}	68.69± 3.14^{b}
Overall	65.13±2.07^{a}	66.06±1.95^{a}	58.31±1.51^{b}	66.83±2.51^{a}	65.32±2.04^{a}	60.22±1.78^{b}
WG (g)	22.08±2.61^{ab}	24.55±2.27^{ab}	19.03±2.16^{b}	29.30±3.46^{a}	22.48±2.28^{ab}	19.11±2.42^{b}
SGR (%)	4.79± 0.18^{b}	4.74±0 .25^{b}	4.82±0.25 ^{b}	5.58±0.21 ^{a}	4.82±0.24 ^{b}	4.83±0.29 ^{b}
FCR (g)	1.92±0.12^{ab}	1. 96±0.30^{ab}	2.19±0.38^{a}	1.34±0.29^{b}	1.61±0.16^{ab}	2.27±0.62^{a}
FI (%/d)	2.55±0.09	2.21±0.12	2.41±0.07	2.28±0.17	2.47±0.15	2.65±0.12

T1: control group, T2: 0.5% garlic oil, T3: 1% garlic oil, T4: 0.5% ginger oil, T5: 1% ginger oil, and T6: 0.5% garlic oil + 0.5% ginger oil.

In the same raw, means ± SE with various letters superscripts is essentially different at ($P < 0.05$).

Table 3: Effect of garlic and/or ginger oil on biochemical parameters of Nile tilapia (means ± SE).

Items	Treatments					
	T1	T2	T3	T4	T5	T6
Thyroid hormones						
T3 (ng/ml)	4.71 ± 0.11	4.88 ± 0.09	4.69 ± 0.12	4.94 ± 0.17	5.03 ± 0.11	4.83 ± 0.15
T4 (ng/ml)	37.28 ± 0.97^{c}	42.87 ± 0.91^{b}	44.54 ± 0.79^{a}	43.03 ± 0.85^{b}	45.12 ± 0.64^{a}	42.67 ± 0.56^{b}
Lipid profile						
Total cholesterol (mg/dl)	150.30 ± 5.42^{a}	121.61 ± 7.44^{b}	96.75 ± 2.55^{c}	127.94 ± 6.71^{b}	118.39 ± 6.22^{b}	98.92 ± 3.23^{c}
LDL cholesterol (mg/dl)	86.95 ± 2.51^{a}	67.21 ± 1.27^{b}	51.62 ± 1.32^{c}	66.78 ± 1.14^{b}	64.78 ± 1.41^{b}	53.83 ± 1.74^{c}
HDL cholesterol (mg/dl)	31.93 ± 0.99^{c}	37.01 ± 0.85^{b}	42.01 ± 0.96^{a}	36.95 ± 1.17^{b}	41.57 ± 1.49^{a}	42.08 ± 1.45^{a}
Kidney function						
Urea (mg/dl)	33.67 ± 0.37^{a}	32.87 ± 0.50^{a}	29.57 ± 0.33^{b}	33.77 ± 0.45^{a}	30.34 ± 0.20^{b}	28.12 ± 0.07^{c}
Creatinine (mg/dl)	0.48 ± 0.01	0.47 ± 0.02	0.50 ± 0.02	0.46 ± 0.02	0.48 ± 0.02	0.47 ± 0.01
Uric acid (mg/dl)	1.88 ± 0.05^{a}	1.44 ± 0.07^{b}	1.29 ± 0.02^{c}	1.40 ± 0.03^{b}	1.31 ± 0.05^{c}	1.26 ± 0.05^{c}
Electrolytes profile						
Calcium (mg/dl)	11.01 ± 0.61	10.89 ± 0.58	10.67 ± 0.35	11.07 ± 0.44	10.55 ± 0.52	10.71 ± 0.39
Phosphorus (mg/dl)	5.98 ± 0.25	6.12 ± 0.31	6.04 ± 0.41	5.93 ± 0.22	6.21 ± 0.33	6.11 ± 0.43

T1: control group, **T2**: 0.5% garlic oil, **T3**: 1% garlic oil, **T4**: 0.5% ginger oil, **T5**: 1% ginger oil, and **T6**: 0.5% garlic oil + 0.5% ginger oil.

In the same raw, means ± SE with various letters superscripts is essentially different at ($P < 0.05$).

(SPSS for Windows, V 25.0; SPSS Inc., Chicago, IL, USA) were used to analyze the data obtained from all variables. The Duncan Multi Range Test of significance was used to determine the significant difference between the treatments means. The data obtained were all stated as mean ±S.E. A level of significance of *P≤ 0.05* or *P≤ 0.01* was viewed as statistically significant.

RESULTS

Growth performance

Data presented in Table (2) revealed that garlic and/or ginger oil supplementation on the fish diet significantly *(P<0.05)* improved the growth performance of Nile tilapia fish. The overall body weight (BW) of fish fed 0.5% garlic oil (T2) and 0.5% and 1% ginger oil (T4 and T5, respectively) was significantly higher than that of T3 and T6 supplemented with 1% garlic oil and a mixture of 0.5% garlic and 0.5% ginger oils. The same results were reported in the second week of supplementation. In the same trend, by the fourth week of the experiment, T4 (0.5% ginger) fish had significantly higher body weight than T3 and T6 fish.

Fish nourished on 0.5% ginger oil added to basal diet (T4) improved the weight gain (WG) of fish with a lower feed conversion ratio (FCR) than 1% garlic oil (T3) and a mixture of 0.5% garlic and 0.5% ginger oils (T6). The specific growth rate of T4 was higher than that of control and other treatments. On the other hand, the diet supplementation with garlic and/or ginger oils significantly *(P>0.05)* had no effect on the feed intake (FI) of all experimental fish.

Biochemical analysis

The administration of garlic oil and/or ginger oil in the Nile tilapia diet at different levels had a significant *(P<0.05)* effect on the levels of T4 hormone in all treatments when related to control group (T1), while these additives significantly (P>0.05) had no effect on T3 hormone levels between treated groups and control one. Conversely, the addition of garlic oil and/or ginger oil at different levels to the Nile tilapia diet markedly *(P<0.05)* decreased the total cholesterol and low-density lipoprotein (LDL-cholesterol) in treated fish in comparison to control group (T1), while these additives markedly *(P<0.05)* improved high-density lipoproteins (HDL-cholesterol) in all treatments as related to the control one (Table 3).

The data of the current study showed in Table (3) concluded that the administration of garlic oil and/or ginger oil to the diet of Nile tilapia at different levels significantly *(P<0.05)* reduced the plasma urea and uric acid concentration in all treatments when compared with the control group (T1). Although these additives significantly *(P>0.05)* had no effect on plasma creatinine, calcium and phosphorus concentration in treated groups when compared to control one.

Relative Expressions of *TNF-a* and *IL-1b* genes

TNF-α and *IL-1β* genes expression was significantly increased in response to supplementation with garlic and ginger oils to fish basal diet ($p < 0.05$) as shown in Figure (1).

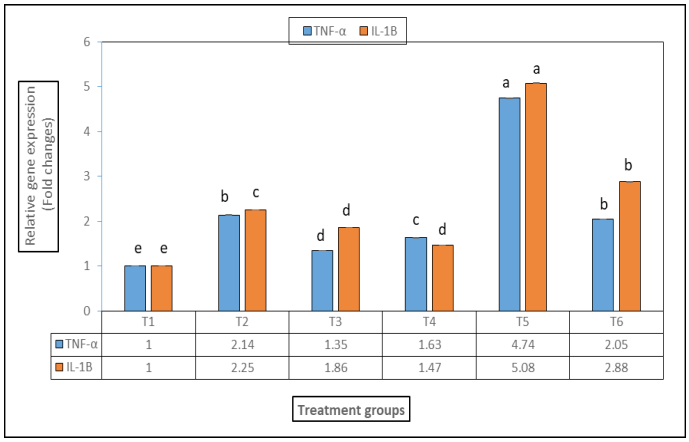

Figure 1: Relative expression of TNF-α and IL-1β genes in spleen tissue of Nile tilapia.
T1: control group, **T2**: 0.5% garlic oil, **T3**: 1% garlic oil, **T4**: 0.5% ginger oil, **T5**: 1% ginger oil, and **T6**: 0.5% garlic oil + 0.5% ginger oil.
Bars with different letters point to significant differences between groups (P < 0.05).

Garlic oil supplementation to basal diet improved the mRNA expression level of *TNF-α* and *IL-1β* gene with better results observed in group supplemented with 0.5% (T2) where the genes expression level was increased by 2.14 and 2.25 respectively than 1% garlic oil (1.35, 1.86 respectively). Likewise, 0.5% ginger oil supplementation (T4) up regulated *TNF-α* and *IL-1β* mRNA expression level by 1.63- and 1.47-fold changes respectively. The peak expression of *TNF-α* and *IL-1β* genes was detected in group supplemented with 1% ginger oil (T5) as fold changes increased by 4.74 and 5.08 respectively. Compared to control group, mixture of 0.5% garlic and 0.5% ginger oil (T6) significantly up regulated the relative *TNF-α* and *IL-1β* gene expression by 2.05 and 2.88 correspondingly.

DISCUSSION

The present study determined the impacts of garlic and/or ginger oils on performance, physiological responses & gene expression of Nile tilapia throughout four weeks of supplementation.

Results from fish performance proved that ginger oil (*Zingiber officinale)* supplementation on fish diets by 0.5% enhanced overall body weight, weight gain and specific growth rate (SGR) with a lower feed conversion ratio (FCR). Ginger oil are identified as tastiness enhancer, which might be reflected on growth of fish, as described for Nile tilapia (da Silva Cardoso et al., 2021). This improvement of fish performance may be related to the active ingredients of ginger such as essential oils, steroids, flavonoids, phenolics,

saponins, tannins, glycosides, and alkaloids which increase the efficiency of feed consumption and subsequent enhanced growth (Jahanjoo et al., 2018). Similarly, ginger contains a terpene compound characterized by its odor and taste, and this compound enhanced growth by increasing FI and FCR (Jolad et al., 2004). Furthermore, ginger stimulates the digestive enzymes secretion as enzymes of pancreas and bile from the liver, resulting in faster food digestion and aiding in the intestinal bacteria stability (Platel and Srinivasan, 2004).

Our findings were in accordance with da Silva Cardoso et al. (2021), who described that 0.57 ml of ginger oil addition on diet of Nile tilapia fish improved growth and FCR. Moreover, Naliato et al. (2021) demonstrated that dietary ginger powder (2.5, 5, 7.5, 10 and 15 g kg^{-1} diet) supplementation throughout 30 days improved *Oreochromis niloticus* growth performance metrics such as final BW, WG, SGR, and FCR. The same results reported in juvenile Black Rockfish *(Sebastes schlegelii)* (Oh et al., 2022), *C. gariepinus* juvenile (Iheanacho et al., 2017) fed on different concentrations of ginger powder (0.25%, 0.5%, 0.75%, 1% of diet; 250, 750, and 1000 mg/35 liters of water, respectively). The ginger supplementation at rate of 5 and 10 g kg^{-1} diet was furthermost suitable for Asian sea bass growth and livability (Talpur et al., 2013).

Although the appetizing, growth-stimulating effects of ginger on fish types, it was assessed that ginger essential oils addition on Nile tilapia feeds at rates of 0.5 %, 1.0 % for 55 days had no progress in fish growth parameters (Brum et al., 2017). Monteiro et al. (2021) reported similar findings in juvenile tambaqui fish supplemented with ginger oil for 60 days. The holding of various percentages of ginger dust in the zebrafish feed for 60 days (Ahmadifar et al., 2019) and Nile tilapia at levels of 0.5% and 1.0% (Şahan et al., 2016) significantly not affect the parameters of growth of these fish. Alternatively, offering a fish diet containing 1.5% ginger powder to tilapia niloticus for 60 days significantly not affect fish weight but decreased WG and SGR with increasing the FCR (Mahmoud et al., 2019).

The findings of the present study concluded that garlic oil addition to fish diets by 0.5% improved the overall body weight. This effect may be regarded as that the garlic oil contains principal compounds such as methyl and allyl-sulphides of allicin that increase appetite, and digestion (Fazllolahzadeh et al., 2011), as well as improve protein synthesis and have a direct bactericidal effect (Citarasu, 2010), which may lead to fish growth stimulation. additionally, when the garlic oil level was elevated (1%) or the combination of ginger oil (0.5%) and garlic oil (0.5%) was used, all the growth parameters (BW, WG, SGR) were decreased with higher FCR. These results might be connected to the destructive impact of high garlic or allicin concentration on fish health and fish growth performance. Also, this might be owing to that the amount of alkyl sulfide which comes to gut, counteracting with normal metabolism and lowering cell division, leading to bad growth and even death (Shakya and Labh, 2014). Additionally, the mode of action of oil combinations must be deciphered before their recommendation as feed additives (Salinas and Magadan, 2017) to avoid the adverse effects.

Nile tilapia (Oreochromis niloticus) grew the best when nourished a food holding 150 mg/kg garlic oil (Metwally, 2009) Adding garlic powder by 3% had an encouraging impact on the performance of growth in *starlet sturgeon* fingerlings (Lee et al., 2014). Moreover, Saleh et al. (2015) revealed that enhancement in BW, WG, and SGR with lowering FCR of sea bass *(Dicentrarcus labrax)* nourished a food containing 10, 20, and 30 g/Kg for 8 weeks. Nevertheless, Mahmoud et al. (2019) concluded that garlic powder supplementation in the Nile tilapia fish diet (1.5%) had an adverse effect on WG and SGR with increasing the FCR. The same results were reported by Xiang and Liu (2002), who concluded that the fish performance was lowered with elevation of allicin level in the diet of red bellied pacu (*Colossoma barchypomum)* fish.

Blood biochemical parameters are important aquaculture measurements that serve as a reflection of fish health and performance (Fazio, 2019). The administration of different levels of garlic oil and/or ginger oil in tilapia niloticus feeds markedly ($P<0.05$) raised the results of T4 hormone in all treatments when compared with control one (T1), while these additives significantly *($P>0.05$)* not affect T3 hormone values between treated groups and control one.

There were relatively few previous studies about the effect of garlic oil and/or ginger oil administration on thyroid hormones of fish in the database to compare with our study. In humans, Mahmoodi et al. (2011) found that the consumption of uncooked garlic for six weeks has a non-significant *($P>0.05$)* effect on thyroid hormone concentrations in hyperglycemic and/or hyperlipidemic individuals. In male albino rats, the supplementation of ginger extract via mouth at a dosage of 750 mg kg^{-1} for six weeks showed a marked *($P<0.05$)* elevation in serum T3 and T4 concentrations in the ginger extract-treated group as compared with the chlorpyrifos-treated group (El-Kerdasy et al., 2021). The addition of garlic oil and/or ginger oil to tilapia niloticus feeds at different levels markedly *($P<0.05$)* diminishes the total cholesterol and LDL-cholesterol in all treatments in comparison with the control one (T1), while those additives markedly (*$P<0.05$*) increase HDL-cholesterol in all treatments in comparison

to the control one.

The decrease of total lipid in the serum of Nile tilapia nourished on feeds containing garlic in dissimilar forms may be attributed to the sulfur containing compound of garlic, which improves fish health and may increase the oxidation of plasma and cell lipids (Metwally, 2009).

The current findings are in agreement with those of Zare et al. (2021), who reported that the serum cholesterol was markedly lowered in the garlic-fed groups. Also, the supplementation of garlic powder at a level of 5, 10, 15, and 20 g kg^{-1} diet lowered the triglycerides and cholesterol levels in Asian sea bass (Talpur and Ikhwanuddin, 2012) besides in rainbow trout at 20, 30, and 50 g kg^{-1} (Mohebbi et al., 2012). But, the addition of garlic dust at 10 g kg^{-1} diet elevated the triglycerides and cholesterol levels in sobaity sea bream (Jennings et al., 2016). The chief bioactive complex in garlic is allicin, which is accountable for lowering the blood triglycerides and cholesterol (Liu and Yeh, 2002) and hinders cholesterol production (Yamamoto and Oue, 2006).

The current results agreed with Chung et al. (2021), who revealed that the plasma cholesterol level was negatively correlated with elevating levels of the essential oil of ginger in the tilapia niloticus feed. But those findings were dissimilar with that of Oh et al. (2022), who reported that the plasma cholesterol levels of juvenile Black Rockfish (*S. schlegelii*) weren't affected markedly by dietary ginger juice extraction residue supplementation. This decrease was related to the existence of gingerol and shagol compounds in ginger, which inhibit peroxidation of lipid (Ekuagbere et al., 2018).

The kidney functions could be evaluated by measuring serum creatinine and urea levels (Azu et al., 2010). The current trial revealed that administration of 1% garlic oil (T3) and 1% ginger oil (T5) and both of them (T6) to the diet of Nile tilapia significantly *(P<0.05)* reduces the plasma urea and uric acid concentration in comparison with T1 (control group), T2, and T4. Also, these additives significantly *(P>0.05)* not affect plasma creatinine concentration in all groups in comparison to control one. The decrease in serum urea level confirms that phytobiotics such as garlic or ginger oil may improve digestion, absorption, and consumption of dietary protein, resulting in improved protein utilization with no adverse effect on liver and kidney functions (Oni et al., 2018).

Those findings were similar to that of Oni et al. (2018), who concluded that serum urea concentration was reduced *(P < 0.05)* significantly as phytomix (mixture of garlic, ginger, and chaya leaf) supplementation level increased in the diet of pullet chicks. The present results disagreed with Abdelwahab et al. (2020), who concluded that the concentrations of blood urea nitrogen, uric acid, and creatinine in Asian sea bass nourished garlic and/or ascorbic acid were significantly not affected as compared to the control one.

Minerals are inert elements, existing in whole somatic fluids and tissues, and their occurrence is important for the support of certain physicochemical procedures that are essential for life. The plasma levels of calcium and phosphorus showed a statistically non-significant *(P>0.05)* difference in the garlic oil and/or ginger oil treated-groups in comparison with T1 (control group). Those results were in agreement with Sheriff et al. (2017), who concluded that the ginger supplementation had a non-significant effect on serum calcium levels in the ginger-treated group as compared to control one. Our findings are unrelated to those of Tende et al. (2014), who reported that the ginger extract-treated groups had a significant decrease in serum calcium and sodium concentrations in comparison to the control group.

Immunostimulant administration is one of the utmost vital procedures of disease regulation in aquaculture by improving the resistance mechanisms of fish (Labh, 2020). Modulation of the innate immune response is carried out mainly through the proinflammatory cytokines secreted by immune cells, especially *TNF-α and IL-1β* thus, gene expression patterns of this cytokine could be used to judge changes in an immune response. Modest level of proinflammatory cytokines transcription is advantageous to increase fish resistance against pathogen infection and maintain immunological balance. The findings of the current trial indicated that Nile tilapia fed on garlic oil and/or ginger oil significantly up regulated expression of immune related genes *TNF-α and IL-1β*. Previous studies have proven that fish supplementation with ginger had varied impacts on cytokine expression depended on the circumstance. Fazelan et al. (2020) suggested that supplementation with 10 g ginger/kg mitigated oxidative stress, immunosuppression and improved the defense to disease in common carp reared under high stocking density through up regulating gene expression levels of *TNF-α, IL-1β* and *IL-8*. Ahmadifar et al. (2019) found that feeding ginger at 1–3% had no significant effects on *TNF-α, IL-1β* or *IL8* gene expression. Our findings are also agreed with Tanekhy and Fall (2015) who reported a marked upregulation in the expression level of *TNF- α* in intestine and lymphoid organ of Marsupenaeus japonicus before and after in stimulation with allicin garlic extract. Foysal et al. (2019) detected a slight upregulation of *IL-1β* gene expression in the intestine of Nile tilapia after feed supplementation with garlic. Transcriptomic analysis by

microarrays of fish nourished on garlic, carvacrol, and thymol essential oils containing feeds revealed an increase of genes linked to vesicular transport and exocytosis, biogenesis, oxidation reduction, leukocyte-mediated immunity, and overall metabolism processes (Firmino et al., 2020). Alternatively, Abdel-Tawwab et al. (2021) found a significant up-regulation in the levels of mRNA expression of *IL-1β and IL-4* genes alongside with down regulation of *TNF-α and HSP70* transcripts in response to garlic and chitosan powder treatment against Zearalenone induced toxicity in European seabass.

CONCLUSION

In conclusion, the current results demonstrated that garlic and/or ginger oil supplementation to the fish diet not only enhances growth but also acts as an immunostimulant and improves the general health of Nile tilapia fish. The different concentration of garlic and/or ginger oils boosted the expression of immunity genes (*TNF-α and IL-1β*) with improvement of thyroid hormone (T4) and lowering plasma cholesterol (LDL), urea and uric acid level. Nevertheless, fish diet integrated with 0.5% ginger oils had the most benefits on Nile tilapia fish growth performance as boost the body weight, weight gain, specific growth rate and improve also feed conversion ratio.

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CONFLICT OF INTEREST

No inconsistency of interest has been affirmed by authors.

AUTHORS CONTRIBUTION

Ibrahim S. Abu-Alya: Conceptualization, Methodology, Writing - Original Draft Preparation, Review, and Editing. Enas K. Aziz: Conceptualization, Methodology, Writing - Original Draft Preparation, Review, and Editing. Nagwa I. Sheraiba: Methodology, Writing - Original Draft Preparation, Data curation. Noha A. Osman: Methodology, Data curation. Afaf A. Kishta: Methodology, Review, and Editing. All authors have read and agreed to the published version of the manuscript.

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